

DATA EVALUATION RECORD
AQUATIC INVERTEBRATE LIFE CYCLE TEST
GUIDELINE OPPTS 850.1350 [72-4(C)]

1. **CHEMICAL:** Metconazole

PC Code No: 125619

2. **TEST MATERIAL:** Metconazole technical grade

Purity: 97.9% (83.8% *cis* and
14.1% *trans*)

3. **CITATION**

Authors: Cafarella, M.A.

Title: Metconazole (KNF-S-474m) – Life-Cycle Toxicity Test with
Mysids (*Americamysis bahia*)

Study Completion Date: February 28, 2006

Laboratory: Springborn Smithers Laboratories
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Sponsor: Valent USA Corporation
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Laboratory Report ID: 12709.6236

MRID No.: 468084-23 (Acc. No. 200600069)

DP Barcode: 329169

4. **REVIEWED BY:** Christie E. Padova, Staff Scientist, Dynamac Corporation

Signature: *Christie E. Padova*

Date: 2/27/07

APPROVED BY: Teri S. Myers, Senior Scientist, Cambridge Environmental Inc.

Signature: *Teri S. Myers*

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5. **APPROVED BY:** Sujatha Sankula, OPP/EFED/ERB - I

Signature: *Sujatha Sankula*

Date: 5/1/07

APPROVED BY: Christine Hartless OPP/EFED/ERB - I

Signature: *Christine Hartless* 6-13-07

Date: 6/13/07

6. **STUDY PARAMETERS**

Age of Test Organism: Neonates, ≤ 24 hours old
Definitive Test Duration: 28 days
Study Method: Flow-through
Type of Concentrations: TWA



7. CONCLUSIONS:

Results Synopsis not reported since study classified as invalid

8. ADEQUACY OF THE STUDY**A. Classification: INVALID**

B. Rationale: The negative effect of reproduction in the solvent control group (relative to the negative control group) and the inability to determine a NOAEC based on this parameter resulted in the Invalid classification of this study.

9. MAJOR GUIDELINE DEVIATIONS:

1. The pre-test health of the mysid culture was not reported.
2. Relatively high analytical variability was observed at the nominal 25 µg ai/L treatment level, with measured concentrations exceeding 20% among results (29%).
3. The length of each mysid at the time of sexual discernment was not recorded.
4. Second generation mysids were counted and discarded, whereas OPPTS guidance requires the retainment of offspring, and if possible (before Day 28), the collection of mortality, number of each sex, body lengths, and/or behavior effects data.
5. The photoperiod (16 hours light/8 hours dark) was slightly longer than recommended (14 hours light/10 hours dark), and transition periods were not instituted.
6. The time of first brood release was not included as an endpoint.
7. Percent survival data provided by the study author in Table 3 of the study report could not be verified by the reviewer. The reviewer calculated the total number of surviving organisms from the raw growth data tables provided in Appendix 2 of the study report (e.g., the number of total living organisms on day 28 from rep A of the negative control was 21, as summed from males and females of both replicates on pp. 71 and 72 of the study report). Relative to an initial number of 30 exposed mysids, F0 overall survival is 70% whereas 72% survival is reported on pages 37 and 76 of the study report.
8. Survival was not provided in terms of each gender, except for paired organisms. Although the terminal (day 28) number of surviving males and females could be determined by from raw growth data tables (Appendix 2 of the study report), the number of excess living males and females was not provided at the time of sexual

discernment, and therefore the overall percent mortality for each gender could not be determined by the reviewer.

9. Reproductive success in the solvent group was significantly lower (15%) than that in the negative control group. According to the EPA memo titled, "Interim Policy Guidance for the Use of Dilution-Water (Negative) and Solvent Controls in Statistical Data Analysis for Guideline Aquatic Toxicology Studies", dated March 30, 2006, this deviation resulted in the INVALID classification of this study. Furthermore, there were significant reductions in reproductive success at all treated levels, compared to the negative control; reductions ranged from 12-85%. As a result, a NOAEC could not be determined in this study.

10. MATERIALS AND METHODS:

A. Biological System

Guideline Criteria	Reported Information
Species: An estuarine shrimp species, preferably <u>Americamysis bahia</u> .	<i>Americamysis bahia</i>
Duration of the Test: A mysid test must not be terminated before 7 days past the median time of 1 st brood release in the control treatment.	28 days
Source (or supplier)	In-house cultures maintained by Springborn Smithers Laboratories. The brood stock was originally obtained from Aquatic BioSystems (Fort Collins, CO; date not reported).

Guideline Criteria	Reported Information
Parental Acclimation 1) Parental stock must be maintained separately from the brood culture in dilution water and under test conditions. 2) Mysids should be in good health.	1) Adult mysids were held in artificial seawater as used during preliminary and definitive testing. The seawater in the brood aquaria was characterized as having a salinity of 20-22‰, a pH range of 8.1-8.2, dissolved oxygen saturation of 95-98%, and a specific conductance of 33,000-35,000 $\mu\text{mhos/cm}$ during the 14-day period prior to test initiation. The culture was maintained under a 16 hour light:8 hour dark photoperiod and at a temperature of 26-29°C. 2) Not reported
Parental Acclimation Period At least 14 days	Continuous
Chamber Location: Treatments should be randomly assigned to test chamber locations.	Not reported
Brood Stock: Test started with mysids: 1) from only one brood stock or 2) from brood stock which has not obtained sexual maturity or had been maintained for >14 days in a laboratory with same food, water, temperature, and salinity used in the test.	Mysids used in this test were of similar age (≤ 24 hours old) and from one source (see above description of culture conditions).

Guideline Criteria	Reported Information
<p>Distribution:</p> <p>No. of mysids before pairing: Minimum of 15 mysids per compartment, 2 compartments per chamber, 2 chambers per concentration for a total of 60/treatment level.</p> <p>No. of mysids after pairing: ≥ 20 randomly selected pairs/treatment (excess males should be held in separate compartment in same treatment to replace paired males).</p>	<p>60/level: 15 mysids per retention chamber, 2 chambers per aquarium, and 2 aquaria per treatment level.</p> <p>20 pair/level: 10 mature pair per replicate aquarium. Excess organisms were pooled and retained in one initial retention chamber; males from the pool were used to replace dead males from the paired groups.</p>
<p>Pairing:</p> <p>1) Should be conducted when most of the mysids are sexually mature (usu. 10-14 days after test initiation).</p> <p>2) Should be paired on the same day</p>	<p>1) When the mysids reached sexual maturity, they were redistributed (paired) within the test aquaria.</p> <p>2) Pairing was performed on Day 15.</p>
<p>Feeding:</p> <p>1) Mysids should be fed live brine shrimp nauplii at least once daily.</p> <p>2) 150 live brine shrimp nauplii per mysid per day or 75 twice a day is recommended.</p>	<p>1) Mysids were fed live brine shrimp (<i>Artemia salina</i>) nauplii, ≤ 48 hours old (post-hydration), twice daily. Prior to pairing, at least one of these feedings was supplemented with Selco®, a substance high in saturated fatty acids. Following pairing, the mysids were fed brine shrimp nauplii enriched with Selco® every other day.</p> <p>2) Quantity not reported.</p>
<p>Counts:</p> <p>Live adult mysids should be counted</p> <p>1) at initiation,</p> <p>2) at pairing,</p> <p>3) and daily after pairing.</p> <p>4) Live young must be counted and removed daily.</p> <p>5) Missing or impinged animals should be recorded.</p>	<p>- Live adult mysids were counted daily.</p> <p>- Dead parental mysids and offspring were recorded, removed, and discarded when observed.</p>

Guideline Criteria	Reported Information
Controls: Negative control and carrier control (when applicable) are required.	Negative and solvent control groups were included.

Comments: Experimental test dates were November 29 to December 27, 2005.

The maximum organism loading concentration (based on a maximum average wet weight of 0.0045 g per mature adult mysid) was 0.0027 g/L/day.

B. Physical System:

Guideline Criteria	Reported Information
Test Water: 1) May be natural (sterilized and filtered) or a commercial mixture. 2) Water must be free of pollutants. 3) During the test, difference between highest and lowest measured salinities must be less than 101 (parts per thousand). Should be measured daily. 4) Salinity should be between 15 and 301. 5) pH should be measured at the beginning, end of test and weekly. 6) DO must be measured at each conc. at least once a week. 7) See details in ASTM E-1191.	1) Artificial seawater was prepared using laboratory well water (not further specified) and a commercially prepared salt formula (hw-MARINEMIX®). 2) Periodic analyses for pesticides, PCB's, and toxic metals in the dilution water indicated that none of these compounds were detected at concentrations that are considered toxic (results not provided). 3) - 4) Salinity was measured daily in each replicate aquarium, and ranged from 18 to 221. 5) pH was measured daily in each replicate aquarium, and ranged from 8.0 to 8.2. 6) DO was measured daily in each replicate aquarium, and ranged from 6.6-8.6 mg/L (91-114% saturation).

Guideline Criteria	Reported Information
<p>Test Temperature:</p> <ol style="list-style-type: none"> 1) Measured daily in one chamber and at least 3 times in all chambers. 2) Mean measured temperature for each chamber at test termination should be within 1EC of selected test temperature. 3) Each individual measured temperature must be within 3EC of the mean of the time-weighted averages. 4) For mysid shrimp, 27EC is recommended. 5) Whenever temp. is measured concurrently in more than one test chamber the highest & lowest temp. must not differ by more than 2EC. 	<p>Temperature was measured daily in each replicate aquarium, and continuously in one control vessel.</p> <p>The target temperature was $26 \pm 2^{\circ}\text{C}$. The actual range was 23-27°C.</p>
<p>Photoperiod: Recommend 16L/8D. 14L/10D also acceptable.</p>	<p>16-hour light, 8-hour dark photoperiod [intensity of 55-85 foot candles (590-920 lux)].</p>
<p>Dosing Apparatus:</p> <ol style="list-style-type: none"> 1) Intermittent flow proportional diluters or continuous flow serial diluters should be used. 2) A minimum of 5 toxicant concentrations 3) A dilution factor not greater than 0.5 and controls should be used. 	<ol style="list-style-type: none"> 1) A modified intermittent-flow proportional diluter 2) five toxicant concentrations 3) dilution factor of 0.5
<p>Toxicant Mixing:</p> <ol style="list-style-type: none"> 1) Mixing chamber is recommended but not required; 2) Aeration should not be used for mixing; 3) It must be demonstrated that the test solution is completely mixed before intro. into the test system; 4) Flow splitting accuracy must be within 10%. 	<ol style="list-style-type: none"> 1) - 3) Criteria not delineated in OPPTS 850.1350 guidance. 4) Within 5% of the targeted delivery.

Guideline Criteria	Reported Information
<p>Test Vessels:</p> <ol style="list-style-type: none">1) Material: all glass, No. 316 stainless steel, or perfluorocarbon plastic2) Size: most common - 300x450x150 mm deep with solution depth of 100 mm.3) Should be covered. <p>Test Compartments (within chambers):</p> <ol style="list-style-type: none">1) Size: 250 ml beaker with side cutouts covered with nylon mesh or stainless steel screen. <p>or</p> <p>90 or 140 mm i.d. glass Petri dish bottoms with collars made of 200 - 250 um mesh screen.</p>	<p>Test Vessels:</p> <p>Glass aquariums measuring 39 x 20 x 25 cm were used. Each aquarium was equipped with a glass self-starting siphon drain to ensure solution exchange within the exposure chambers (retention or pairing chambers described below). The solution volume fluctuated from approx. 4 to 7 L. It was not reported if the aquaria were loosely covered.</p> <p>Test Compartments (within chambers):</p> <p>Prior to pairing, each exposure aquarium contained two mysid retention chambers constructed from glass Petri® dishes (10-cm diameter, 2-cm depth) to which 13-cm high collars of Nitex® screen (210-µm) were attached with silicone sealant. The retention chambers were partially submerged in each aquarium; the solution volume fluctuated from 390 to 710 ml (due to siphon drains). Following pairing, pairing chambers (10/aquarium) were glass Petri® dishes (6-cm diameter) to which 13-cm high collars of Nitex® screen (210-µm) were attached with silicone sealant. The solution volume fluctuated from 100 to 180 ml.</p>

Guideline Criteria	Reported Information
Flow Rate: 1) Flow rates should provide 5 to 10 volume additions per 24 hr. 2) Flow rate must maintain DO at or above 60% of saturation and maintain the toxicant level. 3) Meter systems calibrated before study and checked twice daily during test period.	1) 7.6 volume additions/24 hours 2) DO was maintained at $\geq 91\%$ saturation. 3) The function of the diluter system was monitored daily, and a visual check was performed twice each day. The exposure system was in proper operation for 7 days prior to test initiation to allow equilibration of the test substance in the diluter apparatus and exposure vessels.
Aeration: 1) Dilution water should be aerated to insure DO concentration at or near 100% saturation. 2) Test tanks may be aerated.	1) The dilution water was aerated for 48 hours prior to use. 2) No further aeration described.

Comments: The TOC concentration of the dilution water source was 0.78 and 0.17 mg/L for November and December 2005, respectively.

C. Chemical System:

Guideline Criteria	Reported Information
<p>Concentrations:</p> <p>1) Minimum of 5 concentrations and a control, all replicated, plus solvent control if appropriate.</p> <p>2) Toxicant conc. must be measured in one tank at each treatment level every week.</p> <p>3) One concentration must adversely affect a life stage and one concentration must not affect any life stage.</p> <p>4) The measured conc. of the test material of any treatment should be at least 50% of the time-weighted average measured conc. for >10% of the duration of the test.</p> <p>5) The measured conc. for any treatment level should not be more than 30% higher than the time-weighted average measured conc. for more than 5% of the duration of the test.</p>	<p>1) Five concentration levels plus dilution water control and solvent control levels were all maintained in duplicate.</p> <p>2) Toxicant concentrations were measured in replicate B of each treatment and control level on days 0 and 7, and from alternate replicate test solutions of each treatment and control level on days 14, 21, and 28.</p> <p>3) Criteria met.</p> <p>4) - 5) Relatively high analytical variability was observed at the nominal 25 µg ai/L treatment level, with measured concentrations exceeding 20% among results (29%). The relative variability was ≤20% at all remaining levels.</p>
<p>Solvents:</p> <p>1) Should not exceed 0.1 ml/L in a flow-through system.</p> <p>2) Following solvents are acceptable: triethylene glycol, methanol, acetone, ethanol.</p>	<p>1) 1.0 µL/L</p> <p>2) Acetone</p>

Comments: A preliminary 28-day flow-through exposure was conducted with two replicates per level of 30 mysids/replicate/level (<24 hours old) and nominal concentrations of 0 (negative control), 19, 38, 75, 150, and 300 µg ai/L. Every other day, diluter stock solution was prepared at 4.0 mg ai/L in 20‰ artificial seawater; analytical measurements of both new and aged (48 hours old) diluter stock solutions averaged 50% of nominal concentrations. Therefore, the actual nominal concentrations were adjusted based on this recovery rate to approximately 0, 9.4, 19, 38, 75, and 150 µg ai/L. No statistically-significant differences in survival or growth of females were observed at any level compared to controls. Reproduction was statistically-reduced at the 150 µg ai/L level compared to the control (0.18 versus 0.73 offspring/female/day; 74% reduction). In addition, a treatment-related reduction, although not statistically significant, was observed in the 75 µg ai/L nominal level compared to the control (0.41 versus 0.73 offspring/female/day; 44% reduction). Total length of male mysids was the most sensitive parameter, with statistically-significant reductions compared to the control at the 38, 75, and 150

$\mu\text{g ai/L}$ levels (6.8, 6.9, and 6.6 mm versus 7.3 mm, respectively). Male dry weight was also statistically-reduced at the 150 $\mu\text{g ai/L}$ level compared to the control (0.71 versus 0.91 mg, respectively). The NOAEC, based on male body lengths was 19 $\mu\text{g ai/L}$ (nominal concentration).

Prior to the definitive study, co-solvent (acetone) was used to prepare the 4.0 $\mu\text{g ai/L}$ diluter stock. The use of the co-solvent in the stock solution aided in delivery and mixing of the stock solution, and measured concentrations of the diluter stock (prepared with acetone) were close to the nominal concentration (approx. 100% of nominal).

In a method validation study conducted prior to the definitive test, the mean recovery of *cis*- and *trans*- isomers of metconazole from artificial seawater were $101 \pm 6.80\%$ and $103 \pm 6.80\%$, respectively.

Test water samples were analyzed for residues of *cis*- and *trans*-metconazole using gas chromatography with nitrogen phosphorous detection (GC/NPD). The LOQ was 10 $\mu\text{g ai/L}$. Three quality control (QC) samples were prepared at each sampling interval and remained with the exposure solution samples throughout the analytical process. The QC samples were prepared in dilution water at nominal concentrations similar to the exposure concentration range, and results were used to judge the precision and quality control maintained during the analysis of test samples. Recoveries ranged from 91.5-119%.

11. REPORTED RESULTS:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes. This study was conducted in compliance with EPA Good Laboratory Practice Regulations (40 CFR, Part 160) with the following exception: routine water and food contaminant screening analyses.
Controls: 1) Survival of the first-generation controls (between pairing and test termination) must not be less than 70%. 2) At least 75% of the paired 1 st generation females in the controls produced young or 3) The average number of young produced by the 1 st generation females in the control(s) was at least 3.	1) Survival of paired negative control mysids was 95% (both replicates). 2) and 3) 100% of paired 1 st generation negative control females (20/20) produced young. The average number of young produced was 15.2 per female.

Guideline Criteria	Reported Information
<p>Data Endpoints must include:</p> <ol style="list-style-type: none"> 1) Survival of first-generation mysids Female Male 2) Number of live young produced per female 3) Dry weight of each first-generation mysid alive at the end of the test Female Male 4) Length of each first-generation mysid alive at the end of the study Female Male 5) Incidence of pathological or histological effects; 6) Observations of other effects or clinical signs. 	<p>Endpoints evaluated in this study included:</p> <ul style="list-style-type: none"> - Survival of adults at 28 days (gender specific only for paired adults in raw data tables) - Number of offspring produced per female per reproductive day - Gender specific total body length of adults at study termination - Gender specific dry weight of adults at study termination
<p>Raw data included? (Y/N) At a minimum, individual data should be included for:</p> <ol style="list-style-type: none"> 1) Surviving 1st generation % and & mysids. 2) Number of live young produced per female. 3) Individual length measurements of % and & mysids. 4) Individual dry weight measurements for % and & mysids at the end of the test. 	<p>Raw data were generally provided for all endpoints. However, the number of excess living males and females was not provided at the time of sexual discernment, and therefore the overall percent mortality for each gender could not be determined by the reviewer. Survival data (raw) for paired organisms were provided.</p>

Comments: It was reported that the length of time for brood appearance (i.e., gravid females) was noted for all first generation mysids approximately on day 12; however, the time to first brood release was not assessed as a toxicological endpoint.

Effects Data:

Toxicant Conc. (µg ai/L)			Mean # of Young/fem.	Mean # Young/fem./ repro. day	Mortality, 28 days		Mean Total Length (mm)			Mean Dry Weight (mg)		
Nom.	Meas.	TWA ^(a)			% ^(b)	& ^(b)	%	&	% & &	%	&	% & &
Ctrl	<LOQ	<LOQ	15.2	1.11	5	5	7.5	7.6	NR	0.87	1.20	NR
Sol C	<LOQ	<LOQ	12.0	0.98	6	18	7.5	7.5	NR	0.86	1.12	NR
13	11	11	9.7	0.68	5	5	7.4	7.5	NR	0.86	1.22	NR
25	24	24	11.1	0.76	0	0	7.4	7.6	NR	0.82	1.15	NR
50	50	51	6.6	0.38*	20	15	7.6	7.9	NR	0.89	1.16	NR
100	97	96	4.1	0.17*	5	10	7.3*	7.4	NR	0.80*	1.00	NR
200	180	180	4.4	0.28*	6	12	7.2*	7.2*	NR	0.78*	0.91*	NR

NR – Not reported.

^(a) Reviewer-calculated (see attached Excel spreadsheet).^(b) Relative to paired organisms only. As the number of excess living males and females was not provided at the time of sexual discernment, the overall percent mortality for each gender could not be determined by the reviewer.

* Significantly-reduced compared to the solvent control based on Dunnett's Test (reproduction) or compared to the pooled control based on Williams' Test (growth).

Toxicity Observations: No treatment-related effect on parental survival was observed. On day 28, the study author reported that percent survival was 79 and 75% for the negative and solvent control groups, respectively, and 84, 85, 72, 78, and 86% for the mean-measured 11, 24, 50, 97, and 180 µg ai/L levels, respectively. However, these values could not be verified by the reviewer. The 28-day LC₅₀ value was empirically estimated to be >180 µg ai/L, the highest mean-measured concentration.

Brood appearance (i.e., gravid females) was observed in all test levels by day 15; the time to first brood release, however, was not compared for possible treatment-related effects. Reproductive success (the number of young released per female per day) averaged 1.11, 0.98, 0.68, 0.76, 0.38, 0.17, and 0.28 for the negative control, solvent control, 11, 24, 50, 97, and 180 µg ai/L test groups, respectively, and was statistically-reduced (using the Dunnett's Test) at the ≥50 µg ai/L treatment levels compared to the solvent control group. Offspring were not maintained to observe for possible treatment-related effects on mortality, gender production, or growth.

At study termination, total body length and dry weight of surviving males were statistically-reduced at the mean-measured 97 and 180 µg ai/L treatment levels compared to the pooled control (7.3 and 7.2 mm versus 7.5 mm and 0.80 and 0.78 mg versus 0.87 mg, respectively). For surviving females, total body length and dry weight were statistically-reduced at the mean-measured 180 µg ai/L level compared to the pooled control (7.2 versus 7.6 mm and 0.91 versus 1.20 mg, respectively).

Based on statistically-significant reductions in reproductive success as the most sensitive indicator of toxicity, the LOAEC and NOAEC were reported by the study author to be 50 and 24 µg ai/L, respectively. The MATC was estimated to be 35 µg ai/L.

Statistical Results:

Statistical Method: Statistical analyses were performed on the following endpoint: percent survival at day 28 (combined sexes), the average number of offspring per female per reproductive day, and total lengths and dry weights of surviving organisms (gender specific) on day 28. Results were provided in terms of mean-measured concentrations.

Student's t-Test was used to compare the performance of the control with that of the solvent control for each endpoint. **A significant difference was observed for reproductive success, and comparisons were performed using solvent control data.** For all other endpoints, no significant differences were observed, and both sets of control data were pooled for subsequent analyses.

The Shapiro-Wilk's Test was used to determine if data were normally distributed, and Bartlett's Test was used to determine if variances were homogeneous. All endpoints met the assumptions of normal distribution and homogeneity. Mysid survival and growth were compared with the performance of the pooled control data using Williams' Test. Mysid reproduction was evaluated using Dunnett's Test compared to the solvent control data. The NOAEC and LOAEC were assigned based on significance. Analyses were conducted at the 95% level of certainty, except for the Bartlett's and Shapiro-Wilk's Tests, in which the 99% level of certainty was applied. The MATC was calculated as the geometric mean of the NOAEC and LOAEC.

Most sensitive endpoint: Reproductive success (number of offspring/female/reproductive day)

Endpoint	Method	NOAEC	LOAEC	MATC
Survival	Williams' Test	180 µg ai/L	>180 µg ai/L	N/A
Reproduction (offspring/female/ repro. day)	Dunnett's Test	24 µg ai/L	50 µg ai/L	35 µg ai/L
Total length male	Williams' Test	50 µg ai/L	97 µg ai/L	Not reported
Total length female	Williams' Test	97 µg ai/L	180 µg ai/L	Not reported
Dry weight male	Williams' Test	50 µg ai/L	97 µg ai/L	Not reported
Dry weight female	Williams' Test	97 µg ai/L	180 µg ai/L	Not reported

Comments: For reproductive success, Dunnett's Test was deemed the more appropriate statistical method for analysis, as the dose response observed in reproduction was not a true monotonic relationship (Williams' Test assumes the true means follow a monotonic relationship). It should be noted that the study author's analysis compared the treated groups to the solvent control group.

12. REVIEWER'S STATISTICAL RESULTS:

Statistical Method: The reviewer verified the study author's results for percent survival, reproductive success, and male and female length and body weight. In all cases, the solvent control data were compared to the negative control data using a Student's t-test. The only difference detected between the two was for reproductive success, where this endpoint was 15% lower in the solvent control group. For all endpoint comparisons, the reviewer used the negative control group. All data were analyzed to using the Chi-square and Shapiro Wilks tests to determine normality and the Hartley and Bartlett's tests to determine homogeneity of variances. If data satisfied these assumptions, the NOAEC was determined using ANOVA, followed by Dunnett's or William's test (contingent on a dose-dependent response). If data did not satisfy these assumptions (i.e., male body length), the NOAEC and LOAEC were determined using the non-parametric Kruskal Wallis test. These analyses were conducted using Toxstat statistical software.

Most sensitive endpoint: Reproductive success (average number of offspring per female per reproductive day)

Endpoint	Method	NOAEC	LOAEC
Survival	ANOVA, Dunnett's test	179 µg ai/L	>179 µg ai/L
Reproduction (offspring/repro. day)	ANOVA, Dunnett's test	<11 µg ai/L	11 µg ai/L
Total length	Males: Kruskal Wallis Females: ANOVA, Dunnett's test	Males: 179 µg ai/L Females: 96 µg ai/L	Males: >179 µg ai/L Females: 179 µg ai/L
Dry weight	Males: ANOVA, Dunnett's test Females: ANOVA, William's test	Males: 179 µg ai/L Females: 51 µg ai/L	Males: >179 µg ai/L Females: 96 µg ai/L

Comments: The reviewer's analysis and, thus, conclusions differed from the study author's because the reviewer used only the negative control group to compare to the treated groups, whereas the study author used either the pooled or solvent controls. The reviewer's analysis detected significant reductions in reproductive success at all treated levels, so a NOAEC could not be determined in this study. There were no apparent effects on adult survival or male length and male dry weight. Female growth parameters were affected by treatment, with dry weight being more sensitive than length. The negative effect of reproduction in the solvent control group (relative to the negative control group) and the inability to determine a NOAEC based on this parameter resulted in the Invalid classification of this study.

13. REFERENCES:

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APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL ANALYSIS:

percent survival (reported in Table 3)

File: 8423s Transform: NO TRANSFORM

t-test of Solvent and Blank Controls

Ho: GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CTRL) MEAN =	78.5000	CALCULATED t VALUE =	0.4602
GRP2 (BLANK CTRL) MEAN =	75.5000	DEGREES OF FREEDOM =	2
DIFFERENCE IN MEANS =	3.0000		

TABLE t VALUE (0.05 (2), 2) = 4.303 NO significant difference at alpha=0.05

TABLE t VALUE (0.01 (2), 2) = 9.925 NO significant difference at alpha=0.01

percent survival (reported in Table 3)

File: 8423s Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	0.804	2.904	4.584	2.904	0.804
OBSERVED	0	6	0	6	0

Calculated Chi-Square goodness of fit test statistic = 12.7934

Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

percent survival (reported in Table 3)

File: 8423s Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 477.500

W = 0.971

Critical W (P = 0.05) (n = 12) = 0.859

Critical W (P = 0.01) (n = 12) = 0.805

Data PASS normality test at P=0.01 level. Continue analysis.

percent survival (reported in Table 3)

File: 8423s Transform: NO TRANSFORMATION

DP Barcode: 329169

MRID No.: 468084-23 (Acc. No. 200600069)

Bartlett's test for homogeneity of variance

Calculated B statistic = 7.25
Table Chi-square value = 15.09 (alpha = 0.01)
Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 1.00
Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

percent survival (reported in Table 3)
File: 8423s Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	315.417	63.083	0.793
Within (Error)	6	477.500	79.583	
Total	11	792.917		

Critical F value = 4.39 (0.05,5,6)
Since $F < \text{Critical } F$ FAIL TO REJECT H_0 : All groups equal

percent survival (reported in Table 3)
File: 8423s Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 H_0 : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	78.500	78.500		
2	11	84.500	84.500	-0.673	
3	24	85.000	85.000	-0.729	
4	51	71.500	71.500	0.785	
5	96	78.000	78.000	0.056	
6	179	86.000	86.000	-0.841	

Dunnett table value = 2.83 (1 Tailed Value, $P=0.05$, $df=6,5$)

percent survival (reported in Table 3)

DP Barcode: 329169

MRID No.: 468084-23 (Acc. No. 200600069)

File: 8423s

Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2

Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	2			
2	11	2	25.246	32.2	-6.000
3	24	2	25.246	32.2	-6.500
4	51	2	25.246	32.2	7.000
5	96	2	25.246	32.2	0.500
6	179	2	25.246	32.2	-7.500

percent survival (reported in Table 3)

File: 8423s

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	2	78.500	78.500	78.500
2	11	2	84.500	84.500	79.750
3	24	2	85.000	85.000	79.750
4	51	2	71.500	71.500	79.750
5	96	2	78.000	78.000	79.750
6	179	2	86.000	86.000	86.000

percent survival (reported in Table 3)

File: 8423s

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)

TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	78.500				
11	79.750	0.140		1.94	k= 1, v= 6
24	79.750	0.140		2.06	k= 2, v= 6
51	79.750	0.140		2.10	k= 3, v= 6
96	79.750	0.140		2.12	k= 4, v= 6
179	86.000	0.841		2.13	k= 5, v= 6

s = 8.921

Note: df used for table values are approximate when v > 20.

repro success (avg # offspring/female/repro day)

File: 8423r

Transform: NO TRANSFORM

t-test of Solvent and Blank Controls

Ho:GRP1 MEAN = GRP2 MEAN

DP Barcode: 329169

MRID No.: 468084-23 (Acc. No. 200600069)

GRP1 (SOLVENT CRTL) MEAN	=	1.1050	CALCULATED t VALUE	=	5.0990
GRP2 (BLANK CRTL) MEAN	=	0.9750	DEGREES OF FREEDOM	=	2
DIFFERENCE IN MEANS	=	0.1300			

TABLE t VALUE (0.05 (2), 2) = 4.303** SIGNIFICANT DIFFERENCE at alpha=0.05
TABLE t VALUE (0.01 (2), 2) = 9.925 NO significant difference at alpha=0.01

repro success (avg # offspring/female/repro day)

File: 8423r Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	0.804	2.904	4.584	2.904	0.804
OBSERVED	0	6	0	6	0

Calculated Chi-Square goodness of fit test statistic = 12.7934

Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

repro success (avg # offspring/female/repro day)

File: 8423r Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 0.070

W = 0.921

Critical W (P = 0.05) (n = 12) = 0.859

Critical W (P = 0.01) (n = 12) = 0.805

Data PASS normality test at P=0.01 level. Continue analysis.

repro success (avg # offspring/female/repro day)

File: 8423r Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B statistic = 7.15

Table Chi-square value = 15.09 (alpha = 0.01)

DP Barcode: 329169

MRID No.: 468084-23 (Acc. No. 200600069)

Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 1.00

Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

repro success (avg # offspring/female/repro day)

File: 8423r Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	1.225	0.245	20.417
Within (Error)	6	0.070	0.012	
Total	11	1.295		

Critical F value = 4.39 (0.05,5,6)

Since F > Critical F REJECT Ho:All groups equal

repro success (avg # offspring/female/repro day)

File: 8423r Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	1.105	1.105		
2	11	0.685	0.685	3.834	*
3	24	0.760	0.760	3.149	*
4	51	0.380	0.380	6.618	*
5	96	0.170	0.170	8.535	*
6	179	0.285	0.285	7.486	*

Dunnett table value = 2.83 (1 Tailed Value, P=0.05, df=6,5)

repro success (avg # offspring/female/repro day)

File: 8423r Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
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DP Barcode: 329169

MRID No.: 468084-23 (Acc. No. 200600069)

1	neg control	2			
2	11	2	0.310	28.1	0.420
3	24	2	0.310	28.1	0.345
4	51	2	0.310	28.1	0.725
5	96	2	0.310	28.1	0.935
6	179	2	0.310	28.1	0.820

repro success (avg # offspring/female/repro day)

File: 8423r

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	2	1.105	1.105	1.105
2	11	2	0.685	0.685	0.723
3	24	2	0.760	0.760	0.723
4	51	2	0.380	0.380	0.380
5	96	2	0.170	0.170	0.227
6	179	2	0.285	0.285	0.227

repro success (avg # offspring/female/repro day)

File: 8423r

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)

TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	1.105				
11	0.723	3.542	*	1.94	k= 1, v= 6
24	0.723	3.542	*	2.06	k= 2, v= 6
51	0.380	6.714	*	2.10	k= 3, v= 6
96	0.227	8.126	*	2.12	k= 4, v= 6
179	0.227	8.126	*	2.13	k= 5, v= 6

s = 0.108

Note: df used for table values are approximate when v > 20.

males body length

File: 8423ml

Transform: NO TRANSFORM

t-test of Solvent and Blank Controls

Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	7.5000	CALCULATED t VALUE =	0.4472
GRP2 (BLANK CRTL) MEAN =	7.4500	DEGREES OF FREEDOM =	2
DIFFERENCE IN MEANS =	0.0500		

 TABLE t VALUE (0.05 (2), 2) = 4.303 NO significant difference at
 alpha=0.05
 TABLE t VALUE (0.01 (2), 2) = 9.925 NO significant difference at
 alpha=0.01

males body length
 File: 8423ml Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	0.804	2.904	4.584	2.904	0.804
OBSERVED	0	4	4	4	0

 Calculated Chi-Square goodness of fit test statistic = 2.5097
 Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

males body length
 File: 8423ml Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

 D = 0.065

W = 0.877

Critical W (P = 0.05) (n = 12) = 0.859
 Critical W (P = 0.01) (n = 12) = 0.805

 Data PASS normality test at P=0.01 level. Continue analysis.

males body length
 File: 8423ml Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
 Bartlett's test for homogeneity of variance

 These two tests can not be performed because at least one group has
 zero variance.

DP Barcode: 329169

MRID No.: 468084-23 (Acc. No. 200600069)

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

males body length

File: 8423ml

Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	neg control	7.500	7.500	18.000
2	11	7.400	7.400	14.000
3	24	7.400	7.400	14.000
4	51	7.600	7.600	21.500
5	96	7.250	7.250	6.500
6	179	7.200	7.200	4.000

Calculated H Value = 8.868

Critical H Value Table = 11.070

Since Calc H < Crit H FAIL TO REJECT Ho: All groups are equal.

males body length

File: 8423ml

Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 0 6 5 2 3 1 4
6	179	7.200	7.200	\
5	96	7.250	7.250	. \
2	11	7.400	7.400	. . \
3	24	7.400	7.400	. . . \
1	neg control	7.500	7.500 \
4	51	7.600	7.600 \

* = significant difference (p=0.05)

. = no significant difference

Table q value (0.05,6) = 2.936

SE = 3.542

female body length

File: 8423fl

Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

DP Barcode: 329169

MRID No.: 468084-23 (Acc. No. 200600069)

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	0.804	2.904	4.584	2.904	0.804
OBSERVED	0	6	0	6	0

Calculated Chi-Square goodness of fit test statistic = 12.7934
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

female body length

File: 8423fl Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 0.070

W = 0.886

Critical W (P = 0.05) (n = 12) = 0.859

Critical W (P = 0.01) (n = 12) = 0.805

Data PASS normality test at P=0.01 level. Continue analysis.

female body length

File: 8423fl Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B statistic = 2.08

Table Chi-square value = 15.09 (alpha = 0.01)

Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 1.00

Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is
used to calculate the B statistic (see above).

female body length

File: 8423fl Transform: NO TRANSFORMATION

ANOVA TABLE

DP Barcode: 329169

MRID No.: 468084-23 (Acc. No. 200600069)

SOURCE	DF	SS	MS	F
Between	5	0.417	0.083	6.917
Within (Error)	6	0.070	0.012	
Total	11	0.487		

Critical F value = 4.39 (0.05,5,6)

Since $F > \text{Critical } F$ REJECT H_0 : All groups equal

female body length

File: 8423fl

Transform: NO TRANSFORMATION

DUNNETTS TEST

- TABLE 1 OF 2

 H_0 : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	7.650	7.650		
2	11	7.550	7.550	0.913	
3	24	7.650	7.650	0.000	
4	51	7.850	7.850	-1.826	
5	96	7.450	7.450	1.826	
6	179	7.250	7.250	3.651	*

Dunnett table value = 2.83 (1 Tailed Value, $P=0.05$, $df=6,5$)

female body length

File: 8423fl

Transform: NO TRANSFORMATION

DUNNETTS TEST

- TABLE 2 OF 2

 H_0 : Control < Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	2			
2	11	2	0.310	4.1	0.100
3	24	2	0.310	4.1	0.000
4	51	2	0.310	4.1	-0.200
5	96	2	0.310	4.1	0.200
6	179	2	0.310	4.1	0.400

female body length

File: 8423fl

Transform: NO TRANSFORMATION

WILLIAMS TEST

(Isotonic regression model)

TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	2	7.650	7.650	7.675
2	11	2	7.550	7.550	7.675
3	24	2	7.650	7.650	7.675
4	51	2	7.850	7.850	7.675
5	96	2	7.450	7.450	7.450
6	179	2	7.250	7.250	7.250

female body length

File: 8423fl

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)

TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	7.675				
11	7.675	0.231		1.94	k= 1, v= 6
24	7.675	0.231		2.06	k= 2, v= 6
51	7.675	0.231		2.10	k= 3, v= 6
96	7.450	1.851		2.12	k= 4, v= 6
179	7.250	3.703	*	2.13	k= 5, v= 6

s = 0.108

Note: df used for table values are approximate when v > 20.

male body weight

File: 8423mw

Transform: NO TRANSFORM

t-test of Solvent and Blank Controls

Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	0.8650	CALCULATED t VALUE =	0.1240
GRP2 (BLANK CRTL) MEAN =	0.8600	DEGREES OF FREEDOM =	2
DIFFERENCE IN MEANS =	0.0050		

TABLE t VALUE (0.05 (2), 2) = 4.303 NO significant difference at alpha=0.05

TABLE t VALUE (0.01 (2), 2) = 9.925 NO significant difference at alpha=0.01

male body weight

File: 8423mw

Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

DP Barcode: 329169

MRID No.: 468084-23 (Acc. No. 200600069)

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	0.804	2.904	4.584	2.904	0.804
OBSERVED	0	6	0	6	0

Calculated Chi-Square goodness of fit test statistic = 12.7934
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

male body weight

File: 8423mw Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 0.010

W = 0.931

Critical W (P = 0.05) (n = 12) = 0.859

Critical W (P = 0.01) (n = 12) = 0.805

Data PASS normality test at P=0.01 level. Continue analysis.

male body weight

File: 8423mw Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B statistic = 1.55

Table Chi-square value = 15.09 (alpha = 0.01)

Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 1.00

Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is
used to calculate the B statistic (see above).

male body weight

File: 8423mw Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.015	0.003	1.500
Within (Error)	6	0.010	0.002	
Total	11	0.025		

Critical F value = 4.39 (0.05,5,6)

Since $F < \text{Critical } F$ FAIL TO REJECT H_0 : All groups equal

male body weight

File: 8423mw

Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2

H_0 : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	0.865	0.865		
2	11	0.860	0.860	0.112	
3	24	0.820	0.820	1.006	
4	51	0.885	0.885	-0.447	
5	96	0.795	0.795	1.565	
6	179	0.795	0.795	1.565	

Dunnett table value = 2.83 (1 Tailed Value, $P=0.05$, $df=6,5$)

male body weight

File: 8423mw

Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2

H_0 : Control < Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	2			
2	11	2	0.127	14.6	0.005
3	24	2	0.127	14.6	0.045
4	51	2	0.127	14.6	-0.020
5	96	2	0.127	14.6	0.070
6	179	2	0.127	14.6	0.070

male body weight

File: 8423mw

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)

TABLE 1 OF 2

DP Barcode: 329169

MRID No.: 468084-23 (Acc. No. 200600069)

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	2	0.865	0.865	0.865
2		11	0.860	0.860	0.860
3		24	0.820	0.820	0.853
4		51	0.885	0.885	0.853
5		96	0.795	0.795	0.795
6		179	0.795	0.795	0.795

male body weight

File: 8423mw

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model)

TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	0.865				
11	0.860	0.122		1.94	k= 1, v= 6
24	0.853	0.306		2.06	k= 2, v= 6
51	0.853	0.306		2.10	k= 3, v= 6
96	0.795	1.713		2.12	k= 4, v= 6
179	0.795	1.713		2.13	k= 5, v= 6

s = 0.041

Note: df used for table values are approximate when v > 20.

female body weight

File: 8423fw

Transform: NO TRANSFORM

t-test of Solvent and Blank Controls

Ho: GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	1.2050	CALCULATED t VALUE =	0.8566
GRP2 (BLANK CRTL) MEAN =	1.1000	DEGREES OF FREEDOM =	2
DIFFERENCE IN MEANS =	0.1050		

TABLE t VALUE (0.05 (2), 2) = 4.303 NO significant difference at alpha=0.05

TABLE t VALUE (0.01 (2), 2) = 9.925 NO significant difference at alpha=0.01

female body weight

File: 8423fw

Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

DP Barcode: 329169

MRID No.: 468084-23 (Acc. No. 200600069)

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	0.804	2.904	4.584	2.904	0.804
OBSERVED	0	6	0	6	0

Calculated Chi-Square goodness of fit test statistic = 12.7934
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

female body weight

File: 8423fw Transform: NO TRANSFORMATION

Shapiro Wilks test for normality

D = 0.039

W = 0.976

Critical W (P = 0.05) (n = 12) = 0.859

Critical W (P = 0.01) (n = 12) = 0.805

Data PASS normality test at P=0.01 level. Continue analysis.

female body weight

File: 8423fw Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance

Calculated B statistic = 3.21

Table Chi-square value = 15.09 (alpha = 0.01)

Table Chi-square value = 11.07 (alpha = 0.05)

Average df used in calculation ==> df (avg n - 1) = 1.00

Used for Chi-square table value ==> df (#groups-1) = 5

Data PASS homogeneity test at 0.01 level. Continue analysis.

NOTE: If groups have unequal replicate sizes the average replicate size is used to calculate the B statistic (see above).

female body weight

File: 8423fw Transform: NO TRANSFORMATION

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MRID No.: 468084-23 (Acc. No. 200600069)

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.139	0.028	4.000
Within (Error)	6	0.039	0.007	
Total	11	0.179		

Critical F value = 4.39 (0.05,5,6)

Since $F < \text{Critical } F$ FAIL TO REJECT H_0 : All groups equal

female body weight

File: 8423fw

Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2

 H_0 : Control < Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	neg control	1.205	1.205		
2	11	1.215	1.215	-0.120	
3	24	1.150	1.150	0.657	
4	51	1.170	1.170	0.418	
5	96	1.000	1.000	2.450	
6	179	0.930	0.930	3.287	*

Dunnett table value = 2.83 (1 Tailed Value, $P=0.05$, $df=6,5$)

female body weight

File: 8423fw

Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2

 H_0 : Control < Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	neg control	2			
2	11	2	0.237	19.6	-0.010
3	24	2	0.237	19.6	0.055
4	51	2	0.237	19.6	0.035
5	96	2	0.237	19.6	0.205
6	179	2	0.237	19.6	0.275

female body weight

File: 8423fw

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	neg control	2	1.205	1.205	1.210
2	11	2	1.215	1.215	1.210
3	24	2	1.150	1.150	1.160
4	51	2	1.170	1.170	1.160
5	96	2	1.000	1.000	1.000
6	179	2	0.930	0.930	0.930

female body weight

File: 8423fw

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
neg control	1.210				
11	1.210	0.062		1.94	k= 1, v= 6
24	1.160	0.556		2.06	k= 2, v= 6
51	1.160	0.556		2.10	k= 3, v= 6
96	1.000	2.533	*	2.12	k= 4, v= 6
179	0.930	3.398	*	2.13	k= 5, v= 6

s = 0.081

Note: df used for table values are approximate when v > 20.

DP Barcode: 329169

MRID No.: 468084-23 (Acc. No. 200600069)

APPENDIX II: RESULTS OF TWA CALCULATIONS:

Nominal Concentration (ug ai/L)	Time (Day)	Measured Concentration (ug ai/L)	TWA (ug ai/L)
13	0	10	11.250
	7	12	
	14	11	
	21	11	
	28	12	
25	0	20	24.375
	7	23	
	14	23	
	21	28	
	28	27	
50	0	48	50.625
	7	48	
	14	49	
	21	57	
	28	49	
100	0	89	96.13
	7	97	
	14	93	
	21	100	
	28	100	
200	0	170	178.75
	7	180	
	14	160	
	21	200	
	28	180	

APPENDIX III: VERIFICATION OF SURVIVAL CALCULATIONS:**Mysid Survival****Combined Sexes**

Mean-measured Concentration ug ai/L	No. Surviving Day 14	Percent Survival Day 14	No. Surviving Day 28*	Percent Survival Day 28
Control	50	83.3	44	73.3
Solvent control	53	88.3	40	66.7
11	55	91.7	50	83.3
24	60	100.0	46	76.7
50	55	91.7	41	68.3
97	56	93.3	46	76.7
180	57	95.0	50	83.3

*Values derived from terminal growth measurements.

DP Barcode: 329169

MRID No.: 468084-23 (Acc. No. 200600069)

Males

Mean-measured Concentration ug ai/L	Max. No. Paired Day 15	No. Surviving Paired Day 28	No. Surviving Unpaired* Day 28	Percent Survival Paired Day 28
Control	20	19	2	95.0
Solvent control	17	16	5	94.1
11	20	19	3	95.0
24	20	20	1	100.0
50	20	16	2	80.0
97	20	19	2	95.0
180	17	16	13	94.1

*Values derived from terminal growth measurements.

Females

Mean-measured Concentration ug ai/L	Max. No. Paired Day 15	No. Surviving Females Paired Day 28	No. Surviving Females Unpaired* Day 28	Percent Survival Paired Day 28
Control	20	19	4	95.0
Solvent control	17	14	5	82.4
11	20	19	9	95.0
24	20	20	5	100.0
50	20	17	6	85.0
97	20	18	7	90.0
180	17	15	6	88.2

*Values derived from terminal growth measurements.